Supplemental Information

Effects of Amino Acids on Phosphate Adsorption onto Iron (Oxy)hydroxide Minerals under Early Earth Conditions

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SUPPLEMENTARY TABLES

To ensure that the phosphate concentration in a 1 mL liquid/solid sample was representative of the total phosphate added, triplicate samples of the phosphate stock solutions prior to being added to the mineral were also analyzed. A complete list of experimental conditions is found in Table S1. The average [PO_4] for these solutions was found to be 10.26 mM. This value was compared to the average [PO_4] in the 1 mL samples at each time point which were found to be 10.31 mM for t = 0, 10.19 mM for t = 1 d, and 10.13 mM for t = 7 d samples. The average % differences between samples at t = 0, t = 1 d and t = 7 d and stock solutions were 8.43%, 9.45%, and 10.02%, respectively. The overall average % difference from all time points was 9.30%. Therefore, we can assume that all phosphate is accounted for and that any phosphate not detected in the liquid is adsorbed onto the mineral. All values are summarized in Table S2. However, it is important to note that in about half of the samples, the [PO_4] in the reaction mixture was more than the [PO_4] added and this could be due to colorimetry/experimental error. In order to keep dilutions consistent, all samples were diluted by a factor of 40. Even after dilution, some samples neared the upper colorimetry limits where overestimation was more likely to occur. It is also possible that iron interference in the assay could have given higher readings, but on average there was only a 0.05 mM difference between the [PO_4] in the reaction mixture and the [PO_4] that was added.

Table S1. Complete List of Initial Experimental Conditions

Experiment #	Total # of Repeats	[Fe]*	[PO_4]	[Organic]	pH_M
1	4	10 mM Fe(II)	10 mM	-	9
2	4	10 mM Fe(II)	10 mM	-	6
3	4	10 mM Fe(III)	10 mM	-	9
4	4	10 mM Fe(III)	10 mM	-	6
5	3	10 mM Fe(II)	10 mM	10 mM Cysteine	9
6	3	10 mM Fe(II)	10 mM	10 mM Cysteine	6
7	3	10 mM Fe(III)	10 mM	10 mM Cysteine	9
8	3	10 mM Fe(III)	10 mM	10 mM Cysteine	6
9	3	10 mM Fe(II)	10 mM	10 mM Histidine	9
10	3	10 mM Fe(II)	10 mM	10 mM Histidine	6
11	3	10 mM Fe(III)	10 mM	10 mM Histidine	9
12	3	10 mM Fe(III)	10 mM	10 mM Histidine	6
13	3	10 mM Fe(II)	10 mM	10 mM Arginine	9
14	3	10 mM Fe(II)	10 mM	10 mM Arginine	6
15	3	10 mM Fe(III)	10 mM	10 mM Arginine	9
16	3	10 mM Fe(III)	10 mM	10 mM Arginine	6

*****The total [Fe] added was 10 mM. However, after precipitation by hydroxide, removal of the supernatant would have also taken out any remaining dissolved iron (more so in Fe(II) experiments than Fe(III)). Hence, the total [Fe] remaining during the reaction was always less than 10 mM.

Table S2. Average Total Phosphate Measured in Samples

Repeat	Experiment #	PO_4 detected in stock (mM)	PO_4 detected in total samples (mM) t = 0	PO_4 detected in total samples (mM) t = 1 d	PO_4 detected in total samples (mM) t = 7 d
1	1	8.49	8.88	9.91	10.10
	2		10.79	10.94	9.52
	3		9.97	9.77	9.22
	4		10.94	10.68	10.71
2	1	10.57	10.35	10.01	10.27
	2		10.40	10.05	10.49
	3		10.53	10.08	10.49
	4		11.93	10.38	10.36
3	1	8.57	9.86	10.20	10.26
	2		9.40	10.00	10.30
	3		9.76	9.71	10.44
	4		9.33	10.07	10.84
4	1	10.71	10.73	10.13	10.13
	2		10.63	11.08	10.53
	3		10.89	11.13	9.97
	4		9.89	11.05	5.88
1	5	10.22	9.37	9.32	10.41
	6	11.50	9.70	10.46	10.12
	7		10.51	10.28	10.62
	8		10.26	10.53	10.06
3	5		10.11	7.81	10.42
2	5	12.01	11.13	10.93	10.69
1	9	10.03	9.47	9.41	10.11
	10	10.67	10.66	10.70	10.07
2	9		10.80	10.78	10.57
3	9	9.95	10.65	10.49	10.49
2	10		11.19	10.61	10.50
1	11		10.77	9.17	10.29
	12		10.69	9.91	10.57
1	13	9.66	9.33	9.76	9.44
2	13	10.75	10.78	10.63	10.04
Average		**10.26**	**10.31**	**10.19**	**10.13**
***Average % Difference = 9.30%**			**8.43**	**9.45**	**10.02**

*The average of the average % difference for all time points (% difference = $100 \times (|[PO_{4,experiment}] - [PO_{4,stock}]|)/[PO_{4,stock}]$) from the PO_4 added (i.e., in stock solution) *versus* PO_4 detected (i.e., in the corresponding reaction mixture).

COLORIMETRY TECHNIQUES

All chemicals used were reagent grade and reagents were prepared with Milli-Q water (18.2 MΩ•cm). Phosphate (PO_4) samples were analyzed using a modified version of the molybdenum blue method from He and Honeycutt (2005) where the total assay volume was increased from 1 mL to 2.5 mL. Iron samples were analyzed using a modified method from Aguirre et al. (2021). Samples were analyzed with a Thermo Fisher GENESYS 30 Visible spectrophotometer set to a wavelength of 852 nm for phosphate analysis and 510 nm for iron analysis. The instrument was calibrated with phosphate standards ranging from 0 to 10 ppm prepared from $Na_2HPO_4 \cdot H_2O$ (Mallinckrodt) stock solution and Fe^{2+} standards ranging from 0 to 175 μM prepared from $FeCl_2 \bullet 4H_2O$ (Fisher Scientific) stock solution. Figure S1 summarizes both techniques.

Phosphate colorimetry analysis: Colorimetry was performed to determine the phosphate concentration in the liquid and solid portions of the sample separately; the difference between liquid and solid is indicative of how much had adsorbed onto the mineral. Six 1-mL samples were taken at each time point. To three samples, 0.5 mL of Milli-Q water were added and the samples were centrifuged using a Fisher Scientific accuSpin Micro 17 centrifuge set to 10x1000 min^{-1} for two minutes. The supernatant was analyzed to determine the phosphate in the liquid portion of the 1 mL total sample. To the other three samples, 0.5 ml of 2.5 M HCl were added to dissolve the mineral. These samples were representative of the total concentration of phosphate in the liquid/solid sample. The reagents and procedure are as follows:

- Reagent A: ascorbic acid (0.1 M) and trichloroacetic acid (0.5 M). 0.704 g of ascorbic acid (J.T. Baker) and 3.268 g of trichloroacetic acid (Fisher Scientific) were dissolved in 40 mL of dH_2O. This reagent was discarded after 24 hours and prepared fresh daily.
- Reagent B: ammonium molybdate (0.01 M). 6.2 g of ammonium molybdate (Alfa Aesar) were dissolved in 500 mL of dH_2O.
- Reagent C: sodium citrate (0.1 M), sodium arsenite (0.2 M), and acetic acid (5%). 2.94 g of sodium citrate (Fisher Scientific) and 2.6 g of sodium arsenite (Sigma Aldrich) were dissolved in 5 mL of glacial acetic acid (Fisher Scientific) and 95 mL of dH_2O.
- All reagents were remade once they were completely used up, unless otherwise specified.

All samples were diluted to 0.8 mL to ensure the readings fell within the calibration curve. To each sample, 1 mL of reagent A, 0.2 mL of reagent B, and 0.5 mL of reagent C were added while agitating the vials in between each reagent addition. The total assay volume was 2.5 mL. The samples were set aside for 10 minutes to let the color fully develop. The samples were then transferred to clean cuvettes and analyzed using the UV-Vis. The concentrations were recorded and multiplied by the dilution factor to get the actual phosphate concentration in each sample. The concentrations in the liquid samples were subtracted from the average concentration of the total samples from each set off triplicates to determine the phosphate concentration adsorbed onto the mineral. Concentrations are reported as percentages of phosphate adsorbed onto the mineral.

Iron Colorimetry Analysis: This colorimetry method is only measures Fe^{2+} and therefore ascorbic acid was used to reduce any Fe^{3+} to Fe^{2+}. The same dissolved liquid/solid samples from the phosphate method were used for iron analysis. 0.05 mL from each sample was pipetted into a scintillation vial (Fisher Scientific) and diluted to 1 mL for both Fe^{2+} and Fe(total) analysis. The reagents and procedure are as follows:

- Reagent A: ascorbic acid (0.8 M). 6.925 g of ascorbic acid (J.T. Baker) was dissolved in 50 mL of dH_2O. This reagent was covered with aluminum foil and made fresh weekly as it is light and air sensitive.
    - NOTE: This reagent was only used for Fe (total) samples to reduce Fe^{3+} to Fe^{2+}.
- Reagent B: sodium acetate (1 M). 4.10 g of sodium acetate (J.T. Baker) were dissolved in 50 mL of dH_2O.
- Reagent C: 0.3% 1,10 phenanthroline. 0.15 g of 1,10 phenanthroline monohydrate (J.T. Baker) were dissolved in 50 mL of dH_2O. The solution was stirred and heated until the phenanthroline was fully dissolved.
- All reagents were remade once they were completely used up, unless otherwise specified.

To the Fe (total) samples, 100 µL of reagent A was added to reduce Fe^{3+} to Fe^{2+}. To keep the volumes consistent, 100 µL of dH_2O was added to the Fe^{2+} samples. The samples were set aside for 10 minutes to ensure that all Fe^{3+} was completely reduced. To all samples, 100 µL of 1 M HCl, 100 µL of reagent B, and 2 mL of reagent C were added while agitating the vials in between each reagent addition; the total assay volume was 3.3 mL. The samples were set aside for 10 minutes to let the color fully develop. Once samples were run, the concentrations were recorded and multiplied by the dilution factor to get the final concentration of Fe^{2+} in each sample. The Fe^{2+} concentrations were subtracted from the Fe (total) concentrations to determine Fe^{3+} concentrations. The concentrations were reported as percentages of reduced iron.

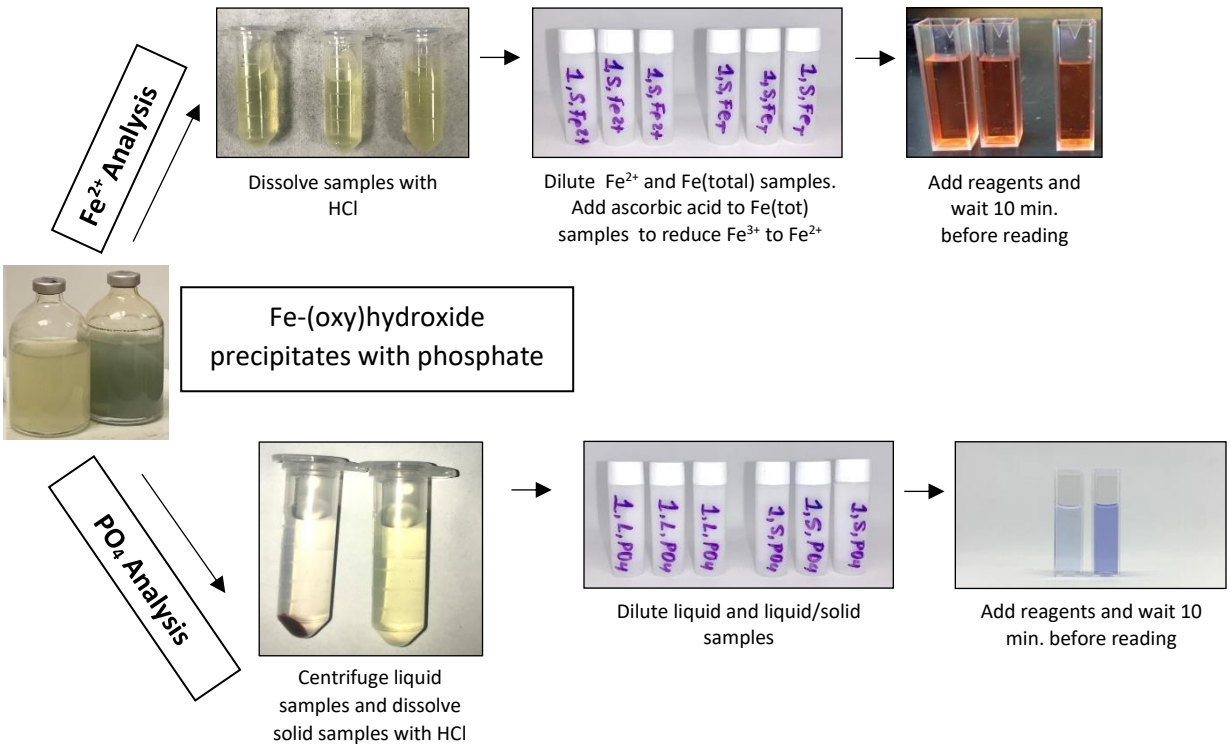

Figure S1. Summary of colorimetry technique for both iron (top) and phosphate (bottom) analysis.

SUPPORTING FIGURES

There was a total of four repeats for PO_4 adsorption controls (absence of AAs) experiments and a total of three repeats for experiments with AAs. Triplicate samples were taken at each time point. Each individual liquid sample was subtracted from the average [PO_4] in a 1 mL sample for each experiment. This value was converted to a percent and plotted as % of PO_4 dsorbed onto the mineral. Therefore, the control plots had a dataset of 12 points for each experiment and the AA plots had a dataset of 9 points for each experiment. Box and whisker plots were created using Veusz program with a whisker mode of 1.5 IQR. The mean is represented by a dot, the median by a horizonal line, and any outliers are represented by an "X".

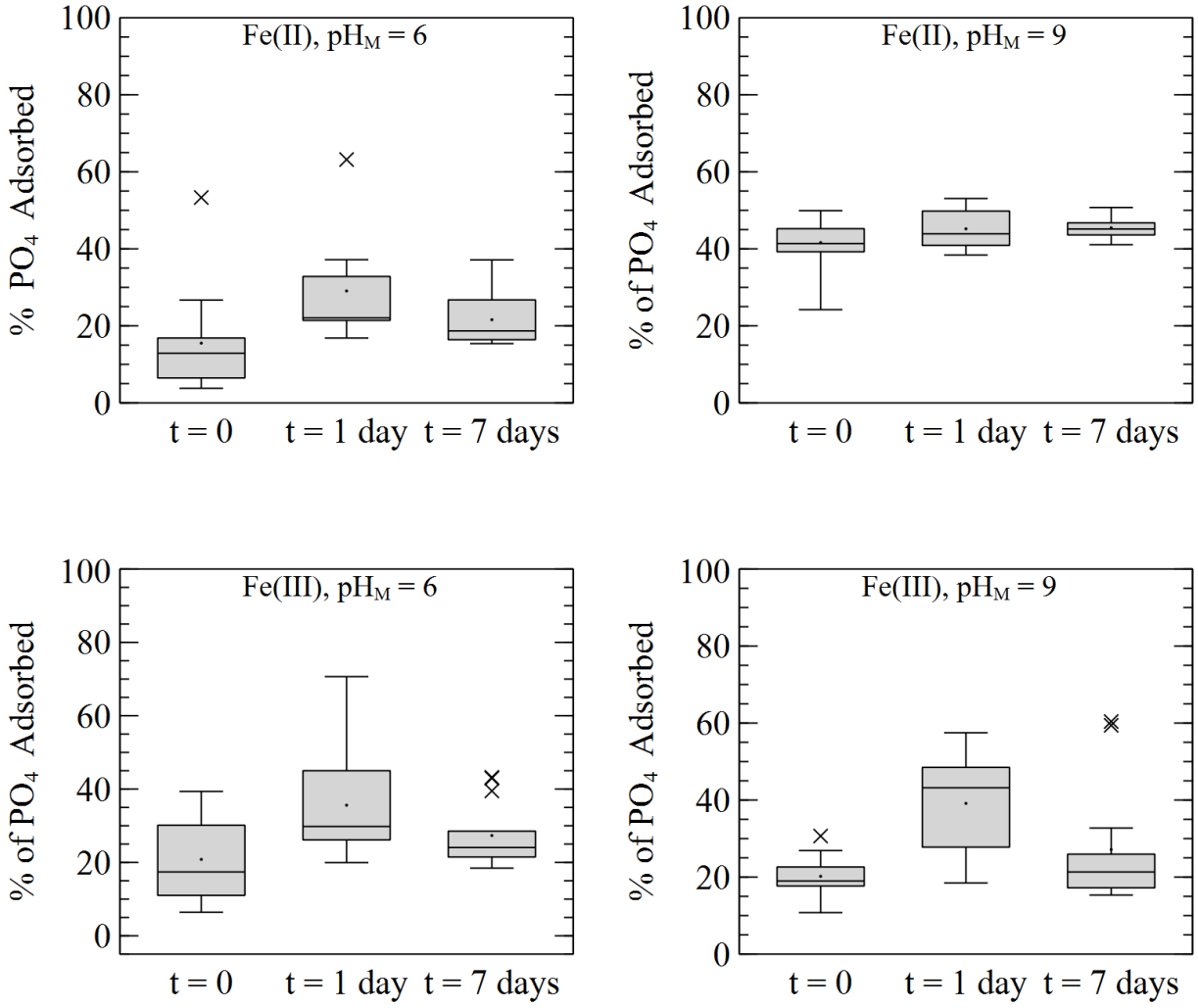

Figure S2. Percent of PO_4 adsorbed onto the mineral in the absence of amino acids at every condition tested and every time point sampled. The mean is represented by the dot and the cross line represents any outliers. Each box and whisker plot was created using 12 data points from four repeats.

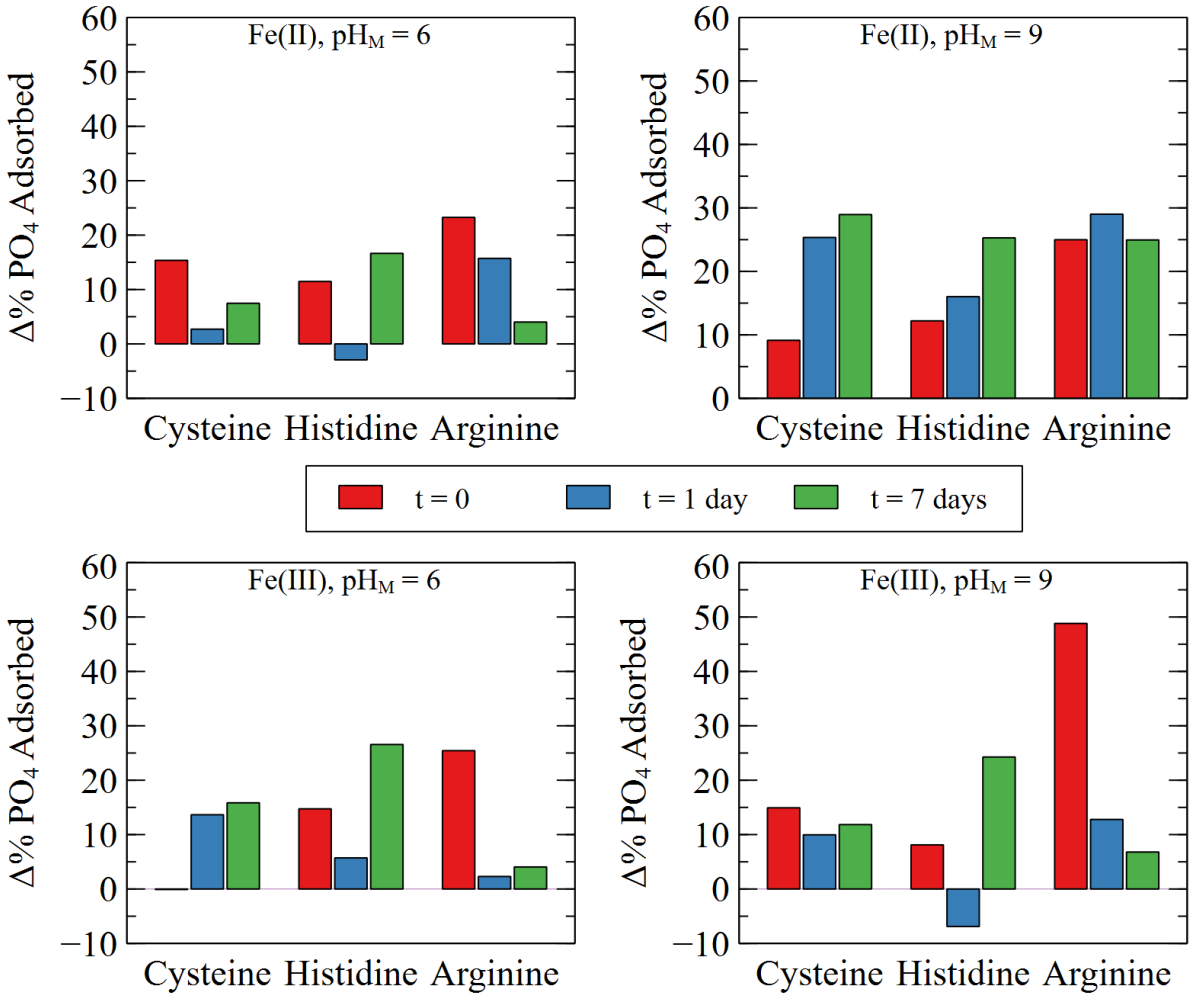

Figure S3. The difference in the averages of phosphate adsorption of control experiments (no AAs) and experiments with amino acids at every condition tested. A positive value denotes an increase in adsorption with the addition of that AA and a negative value denotes a decrease in adsorption when the AA is added.

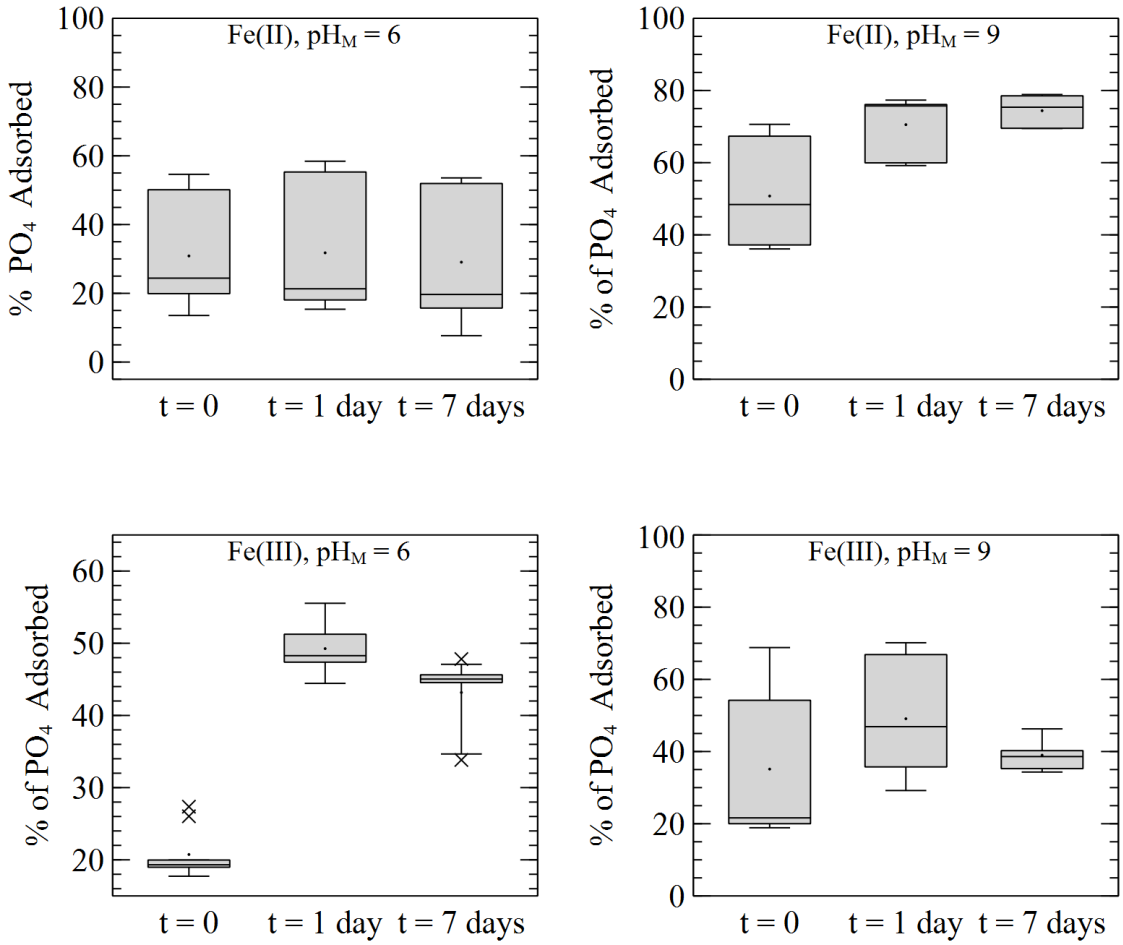

Figure S4. Percent of phosphate adsorbed onto the mineral in the presence of cysteine at every condition tested and every time point sampled. The mean is given by the dot and the cross line represents any outliers. Note the axis for Fe(III), $pH_M = 6$ is altered so the spread of the data is easier to see. Each box and whisker plot was created using 9 data points from three repeats.

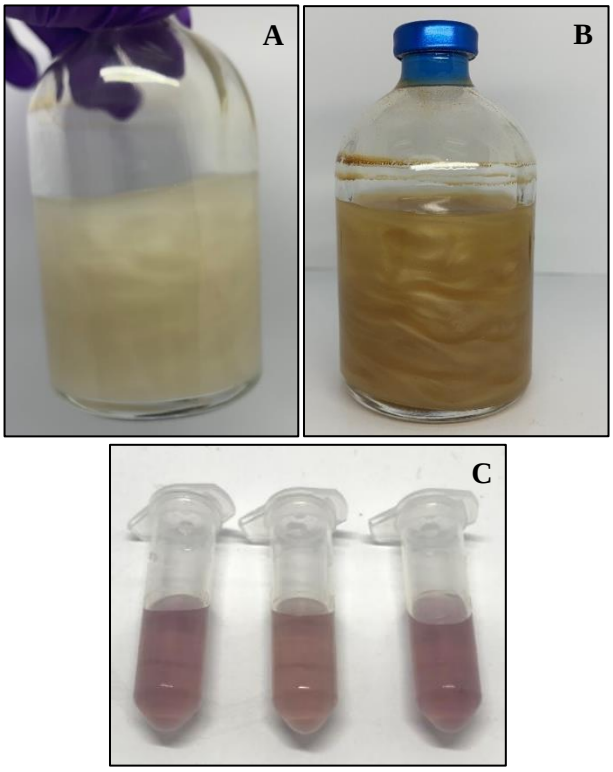

Figure S5. Cysteine solutions at the end of the experiment (t = 7 days) for (A) pH_M = 9 Fe(II) mineral and (B) pH_M = 9 Fe(III) mineral. The height of the bottle is 9.5 cm. (C) Liquid samples exhibited a color change upon vortexing. Color disappeared and sample became clear again after some time.

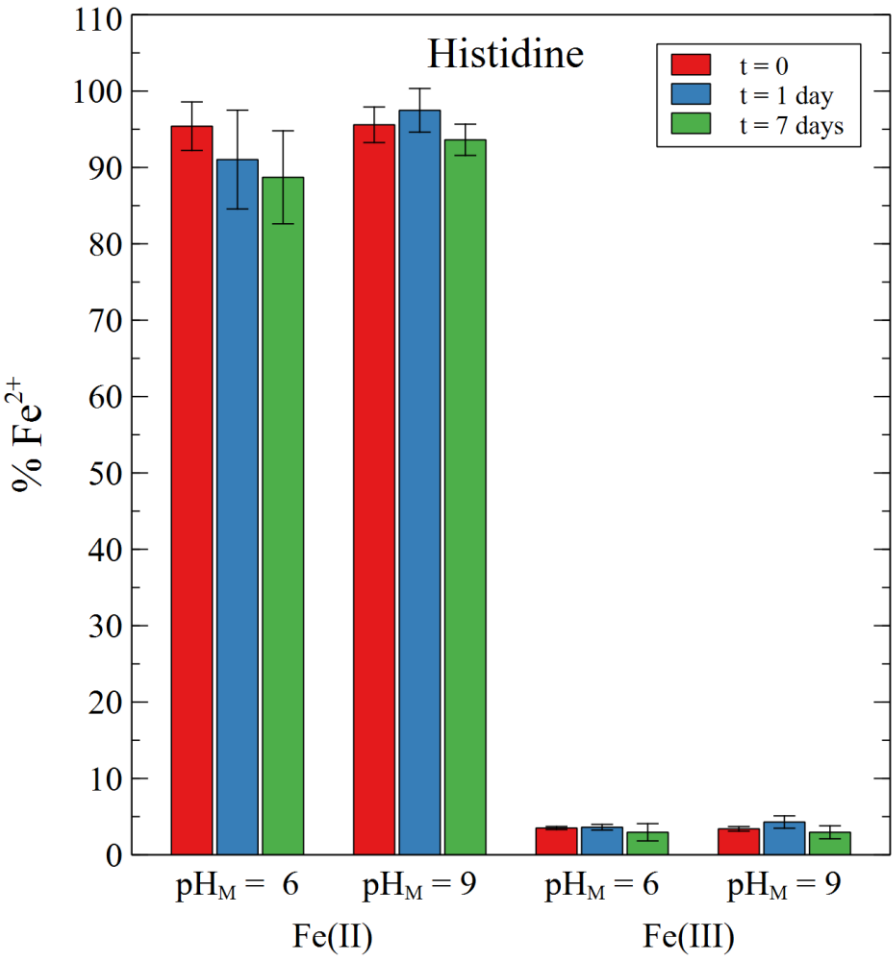

Figure S6. Average percent of Fe(II) detected in a 1 mL sample for histidine at every condition tested.

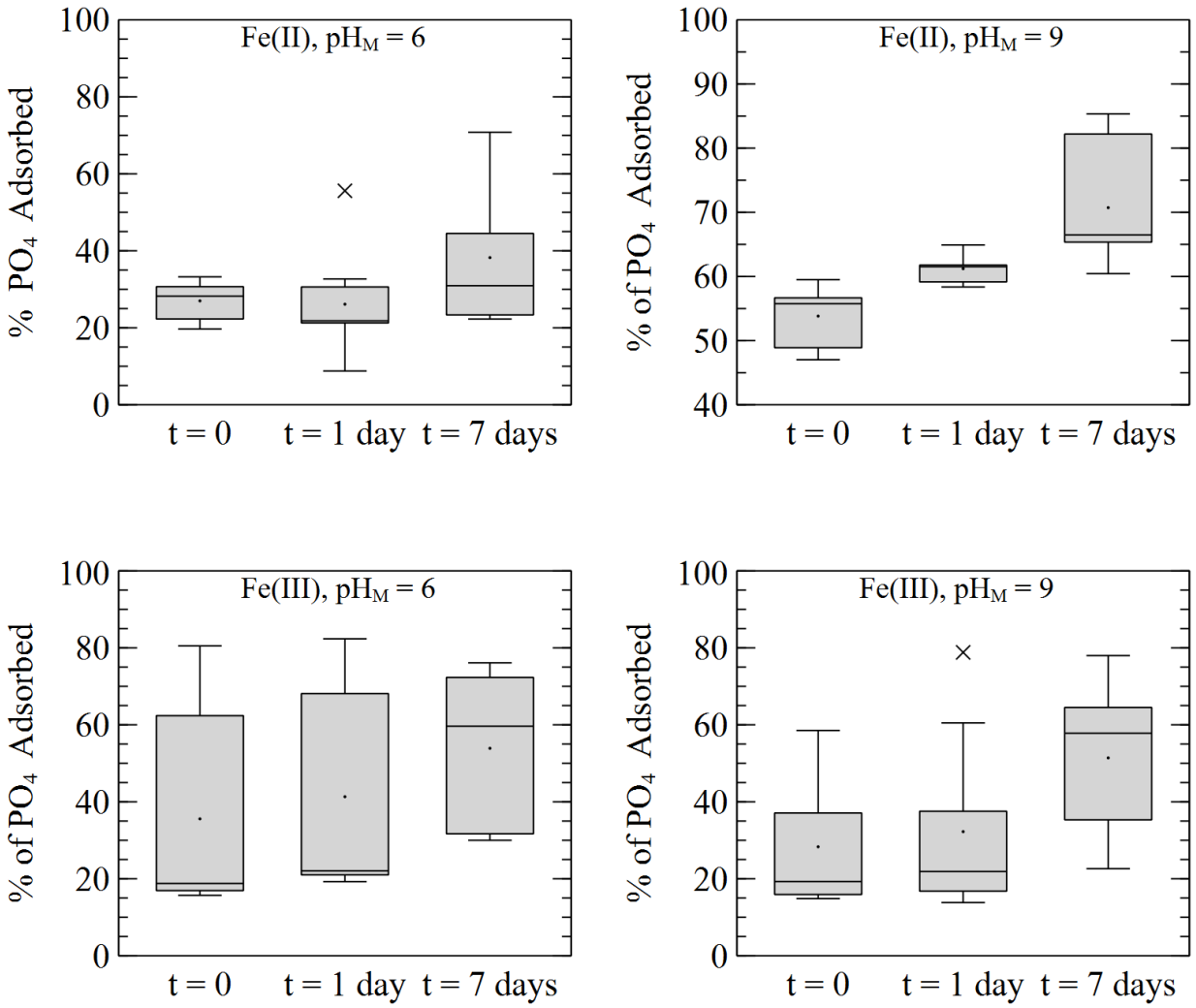

Figure S7. Percent of phosphate adsorbed onto the mineral in the presence of histidine at every condition tested and every time point sampled. The mean is given by the dot and the cross line represents any outliers. Note the axis for Fe(II), $pH_M = 9$ is altered so that the spread of the data is easier to see. Each box and whisker plot was created using 9 data points from three repeats.

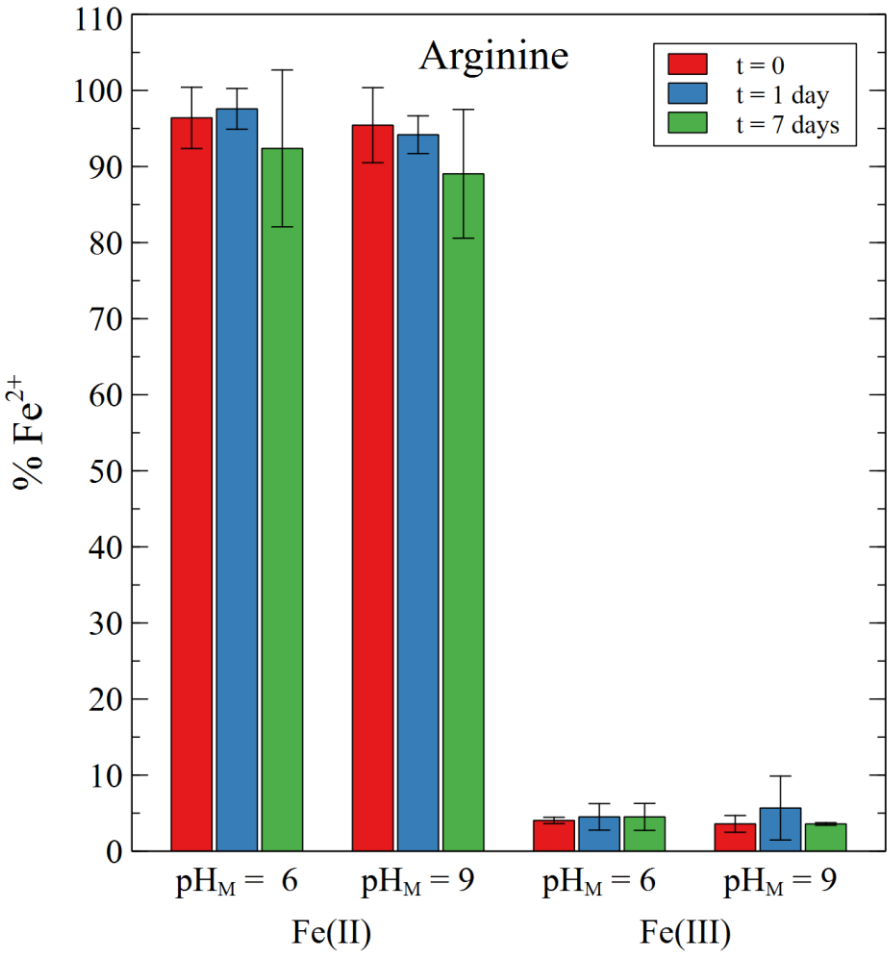

Figure S8. Average percent of Fe(II) detected in a 1 mL sample for arginine at every condition tested.

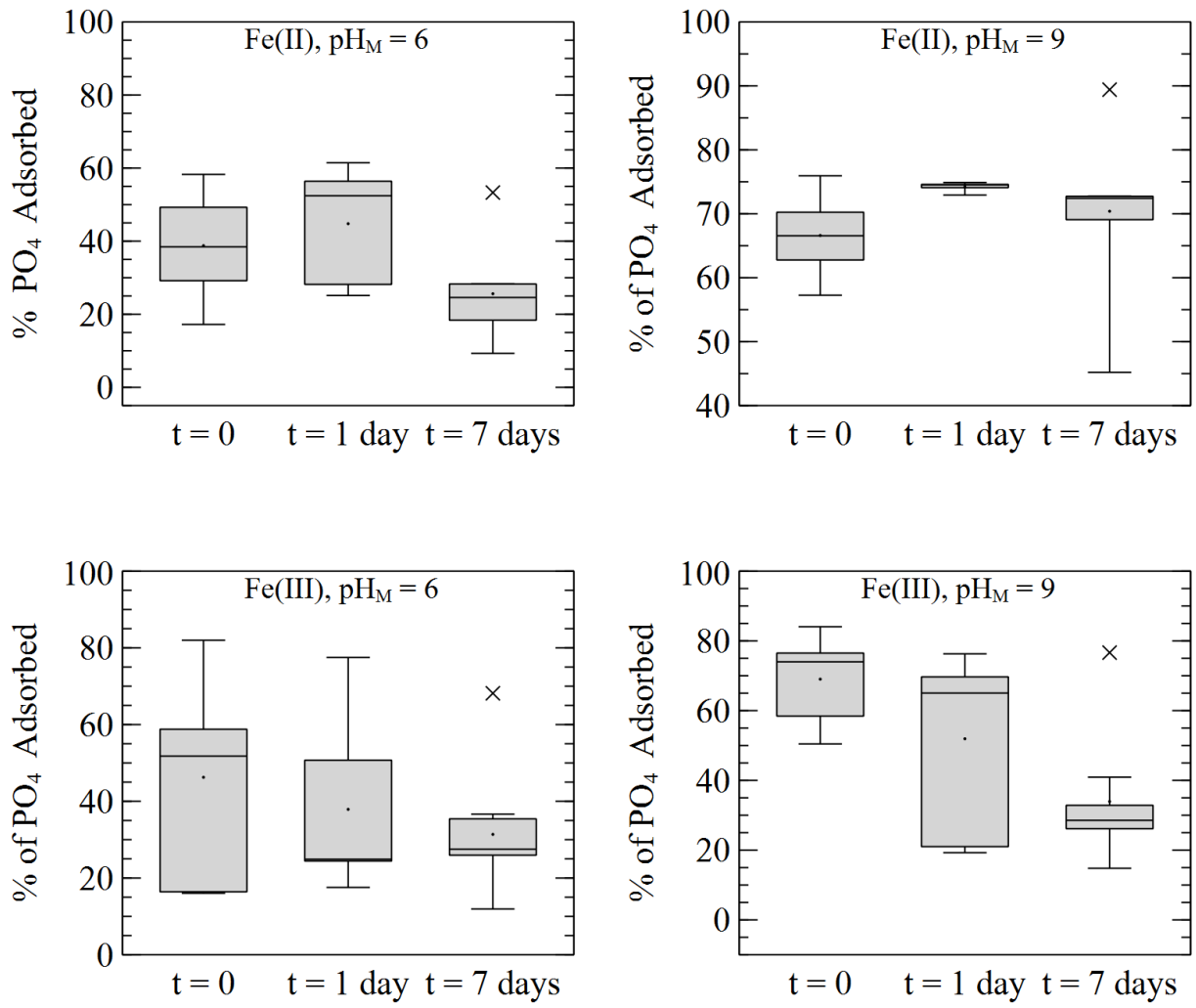

Figure S9. Percent of phosphate adsorbed onto the mineral in the presence of arginine at every condition tested and every time point sampled. The mean is given by the dot and the cross line represents any outliers. Note the axis for Fe(II), pH_M = 9 is altered so that the spread of the data is easier to see. Each box and whisker plot was created using 9 data points from three repeats.

NMR ANALYSIS

^{1}H and ^{31}P NMR analysis was performed to aid in species identification; NMR analysis was not used for quantification. Extra 1-mL aliquots were taken from each experiment for NMR analysis. To each sample, 0.5 mL of 1 M NaOH was added to precipitate out the iron and the sample was centrifuged; the supernatant was used for NMR analysis. Phosphate samples were prepared with 10% ADP/D_2O and the ^{1}H samples were prepared with 10% DSS/D_2O. NMR standards of phosphate, cysteine, histidine and arginine were analyzed to identify the phosphate and amino acid peaks to compare to the experimental spectra. Samples were dropped off at Caltech and run on a 400 MHz Bruker equipped with an autosampler. MestreNova NMR processing software was used to analyze the resulting data. ^{1}H NMR spectra were referenced to DSS (0 ppm) and ^{31}P NMR spectra were referenced to the ADP standard (-6 and -10.5). The water peak was removed from the ^{1}H spectra for clarity. Peak picking tool was used to identify all peak values and automatic multiplet analysis was used for peak area comparison between samples. Sample spectra were superimposed with their corresponding standards to directly compare the control samples to our reactions.

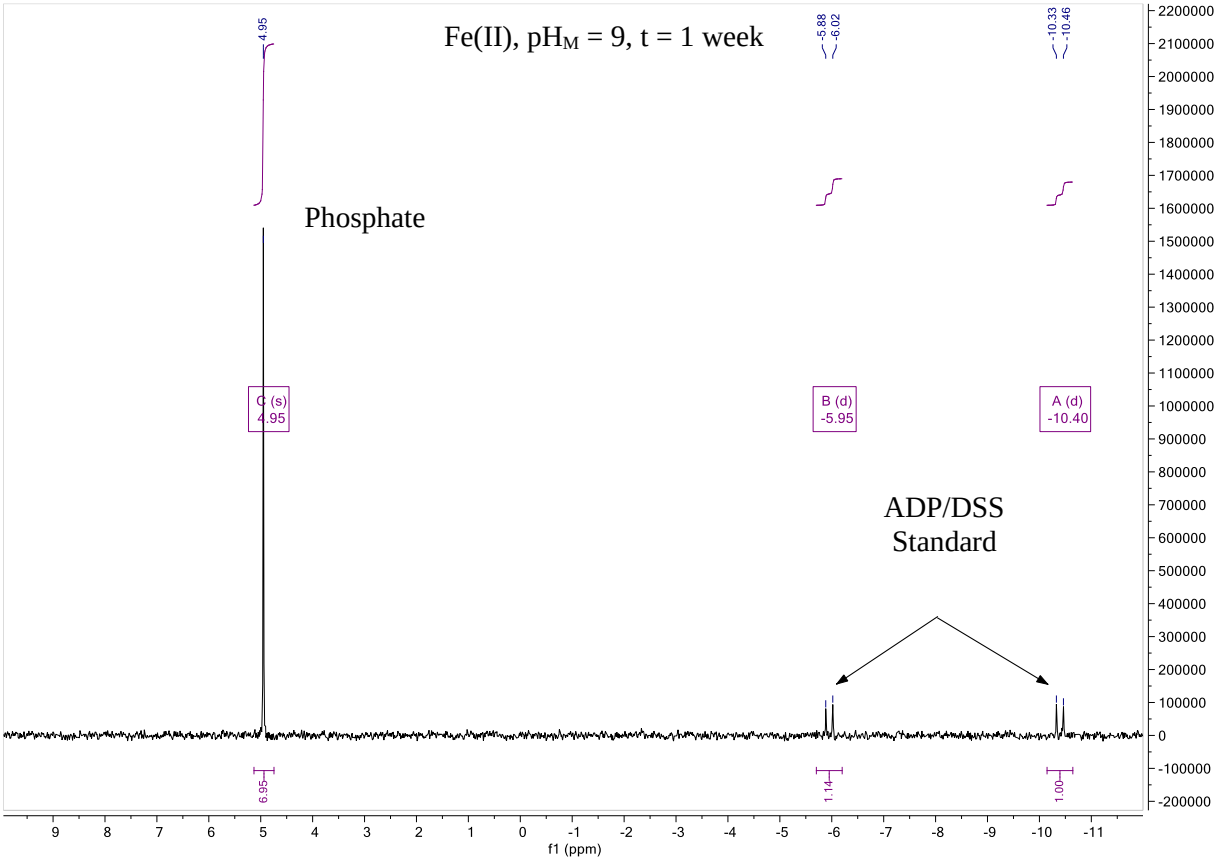

Figure S10. Spectra of ^{31}P NMR for 100% Fe(II), pH$_M$ = 9, t = 1 week.

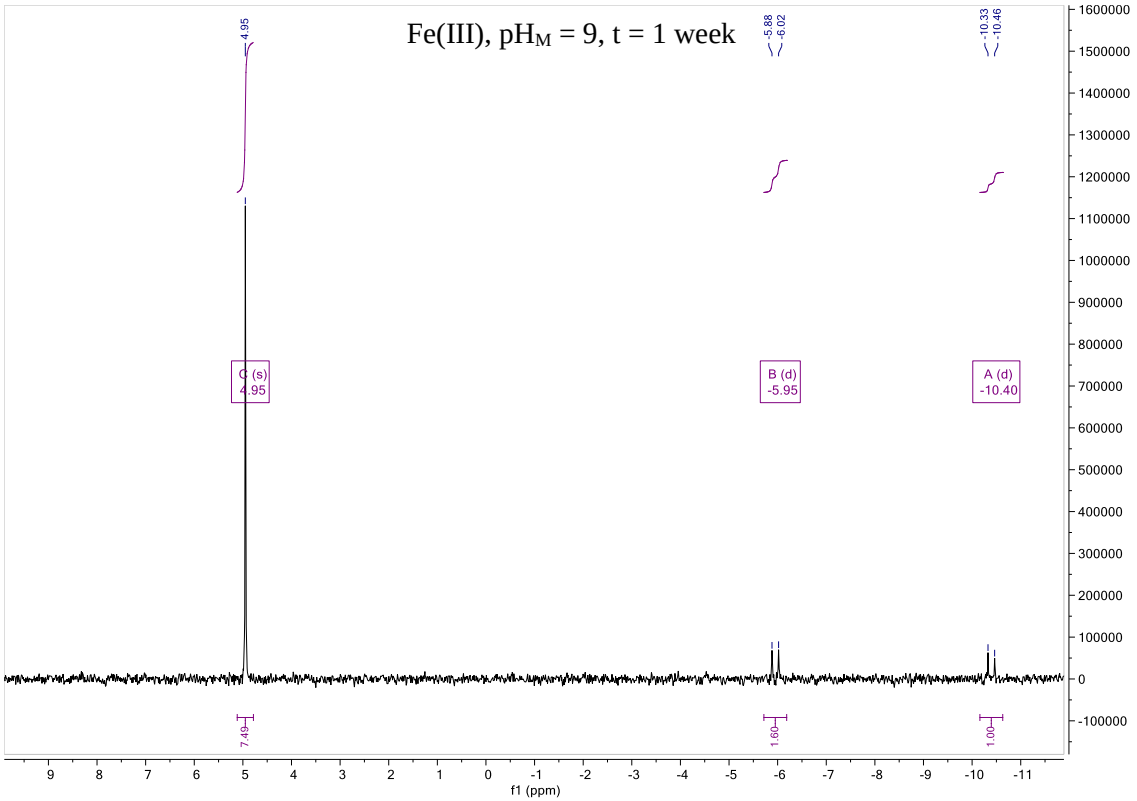

Figure S11. Spectra of ^{31}P NMR for 100% Fe(III), pH$_M$ = 9, t = 1 week.

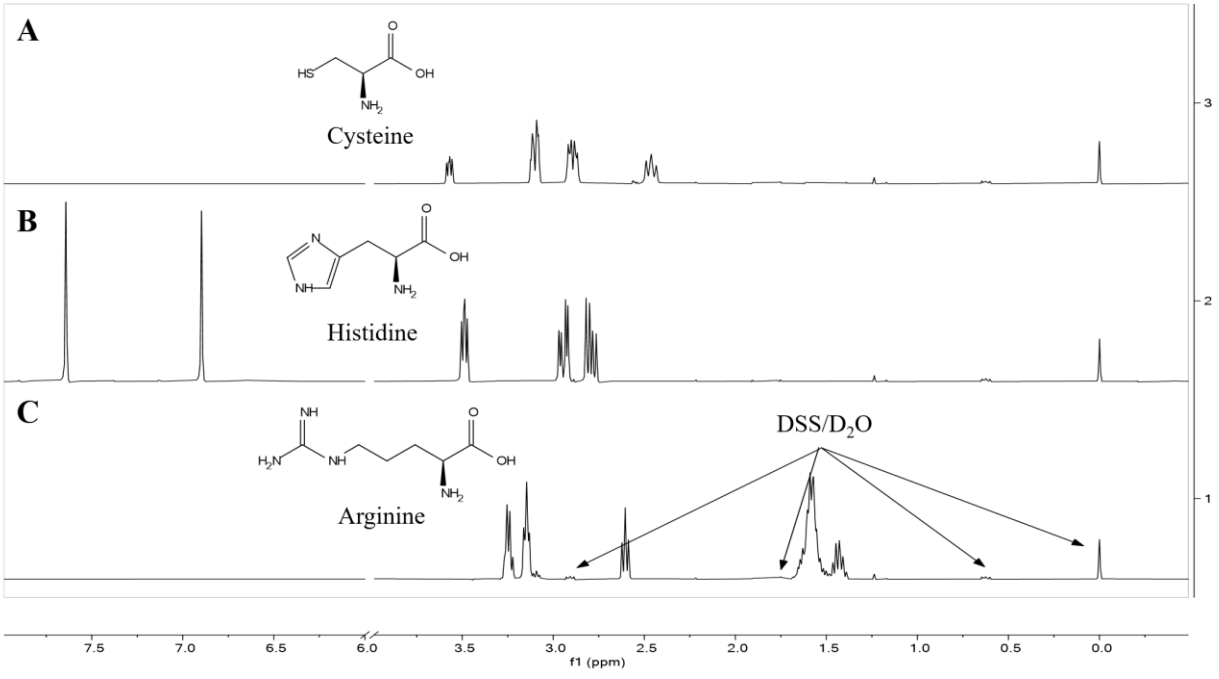

Figure S12. Spectra of ^{1}H NMR for 100% Fe(II) experiments of phosphate with (A) cysteine, (B) histidine (C) arginine at pH$_M$ 9, sampled at 1 week.

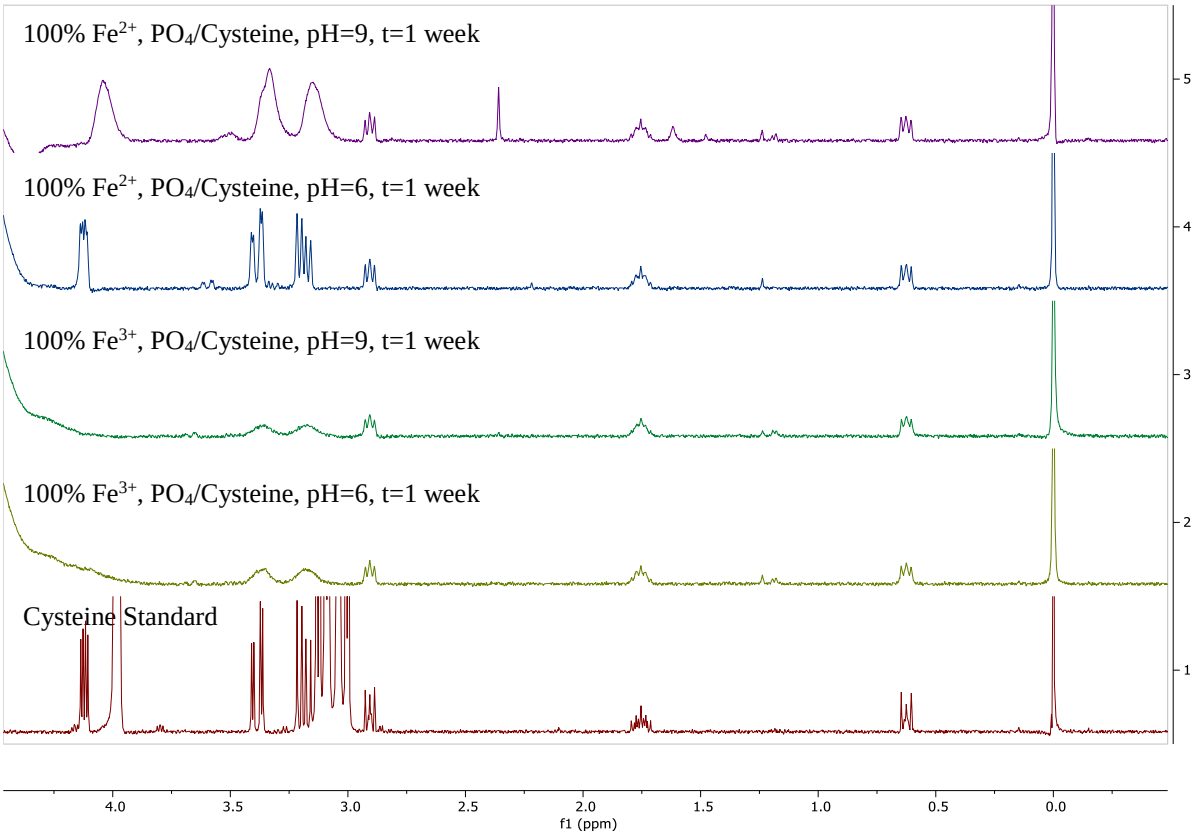

Figure S13. NMR spectra of cysteine experiments at every condition tested for t = 1 week and spectrum of cysteine standard.

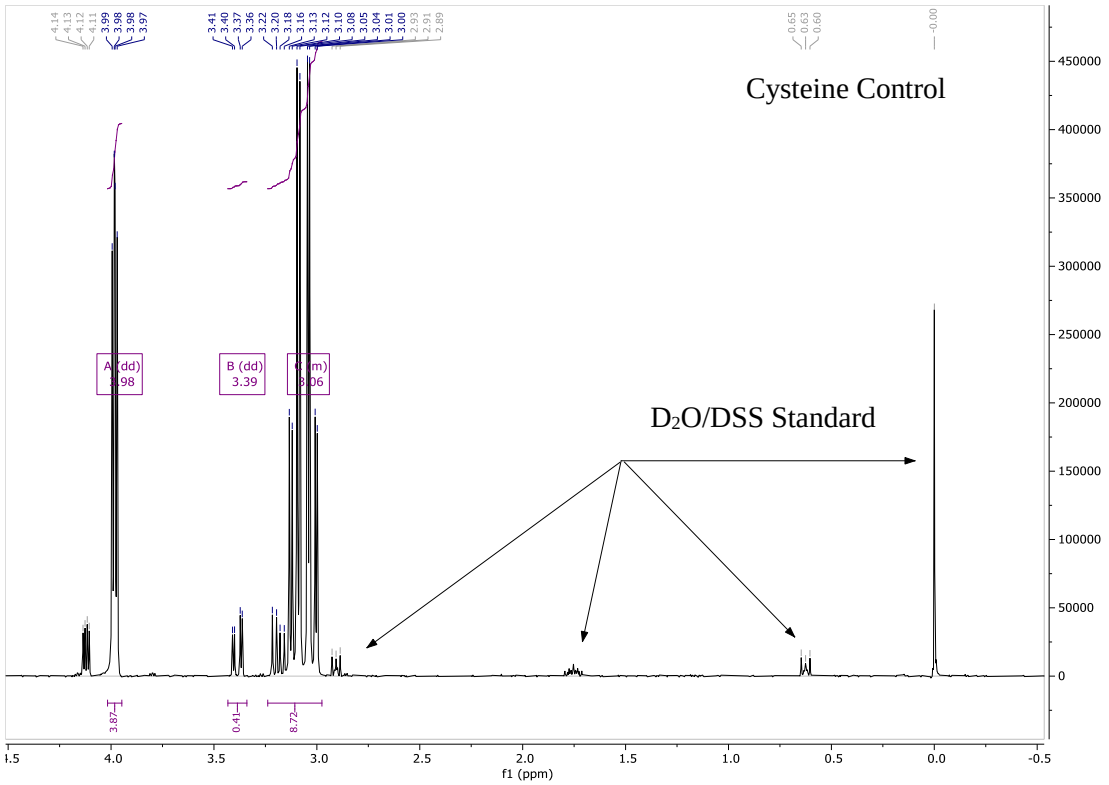

Figure S14. Cysteine control in D_2O/DSS.

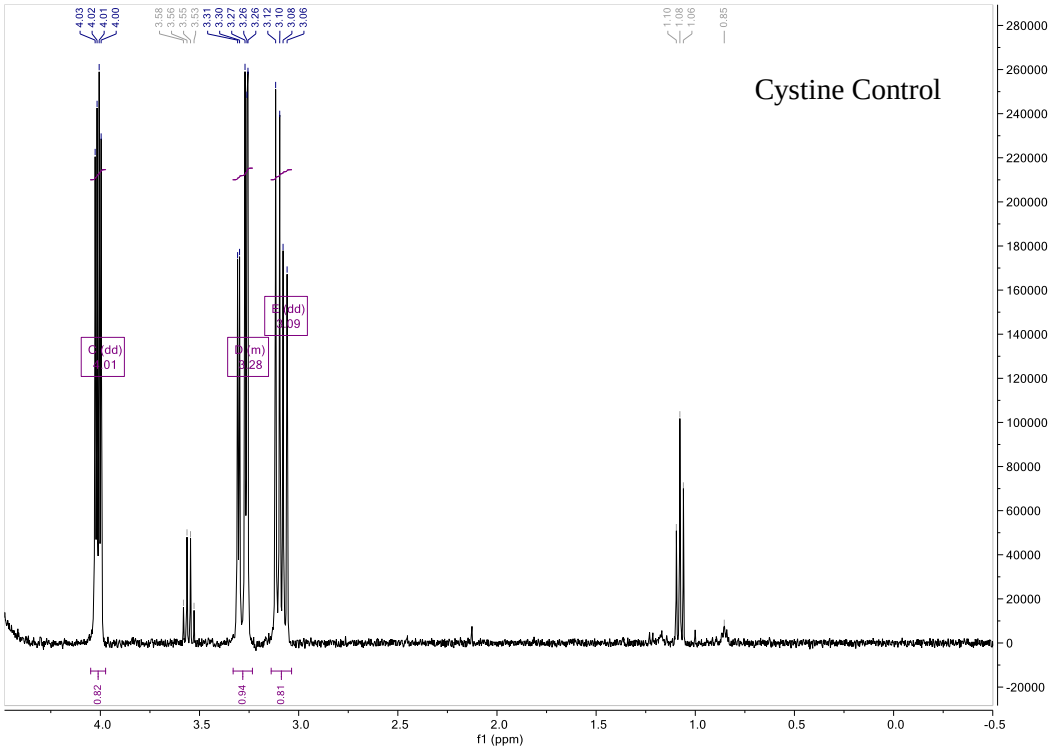

Figure S15. Cystine control at pH 7.

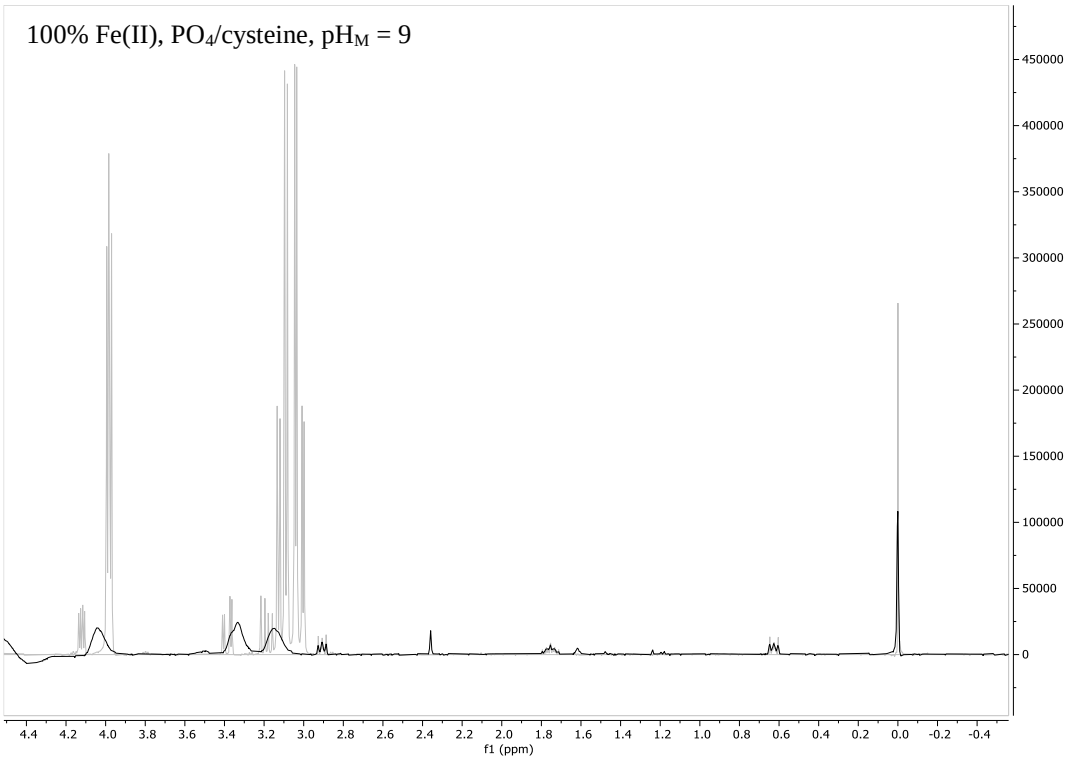

Figure S16. NMR spectra of 100% Fe(II), PO_4/cysteine, pH_M = 9 experiment (black) superimposed onto the cysteine standard (gray).

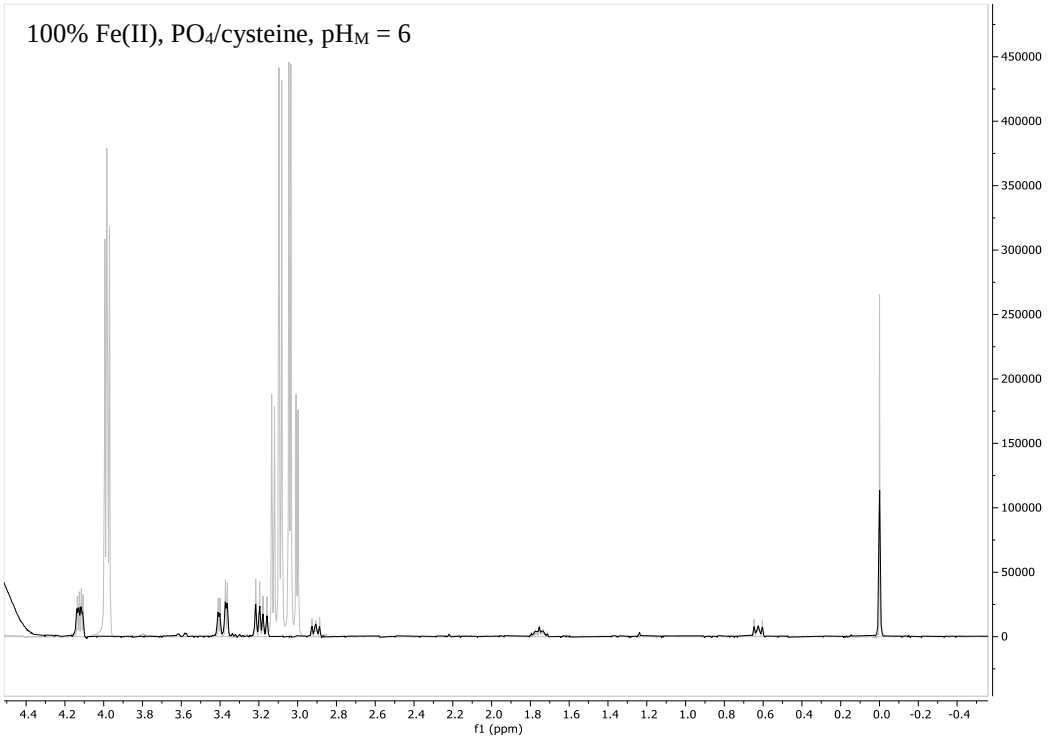

Figure S17. NMR spectra of 100% Fe(II), PO_4/cysteine, pH_M = 6 experiment (black) superimposed onto the cysteine standard (gray).

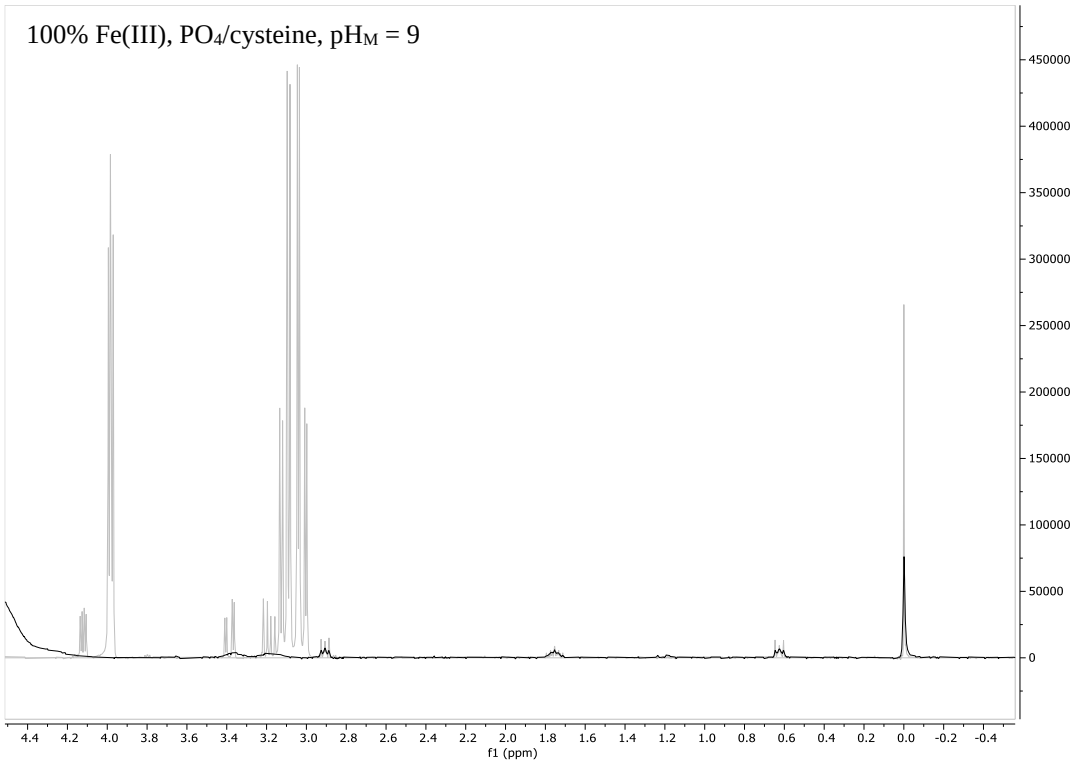

Figure S18. NMR spectra of 100% Fe(III), PO_4/cysteine, pH_M = 9 experiment (black) superimposed onto the cysteine standard (gray).

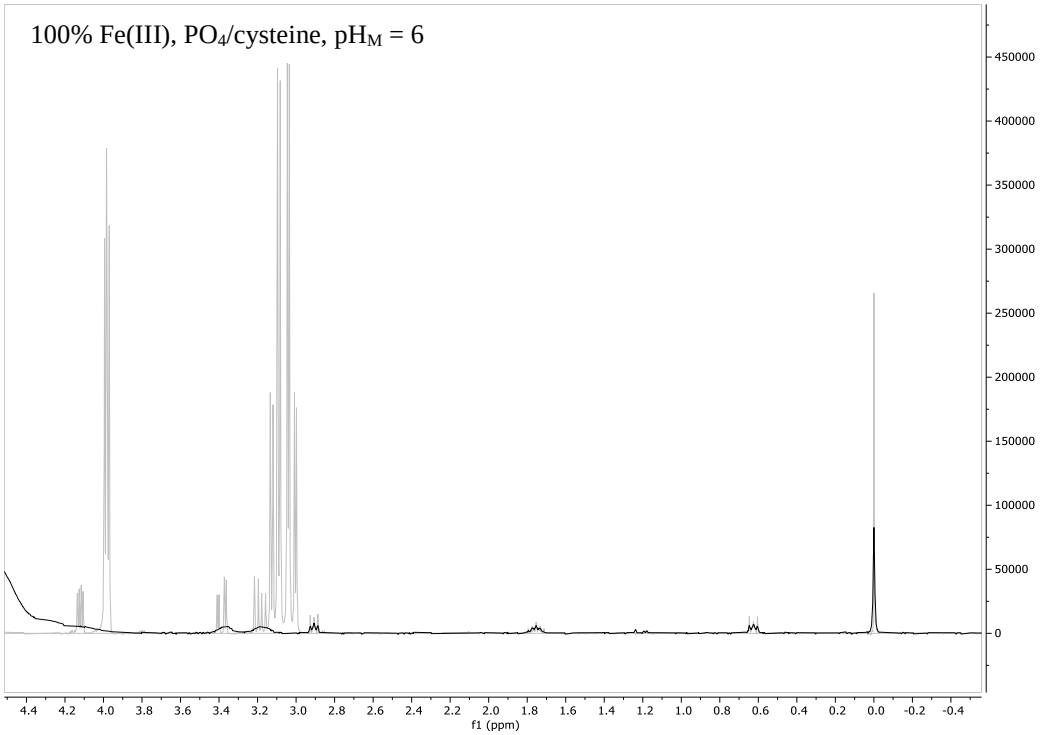

Figure S19. NMR spectra of 100% Fe(III), PO_4/cysteine, pH_M = 6 experiment (black) superimposed onto the cysteine standard (gray).

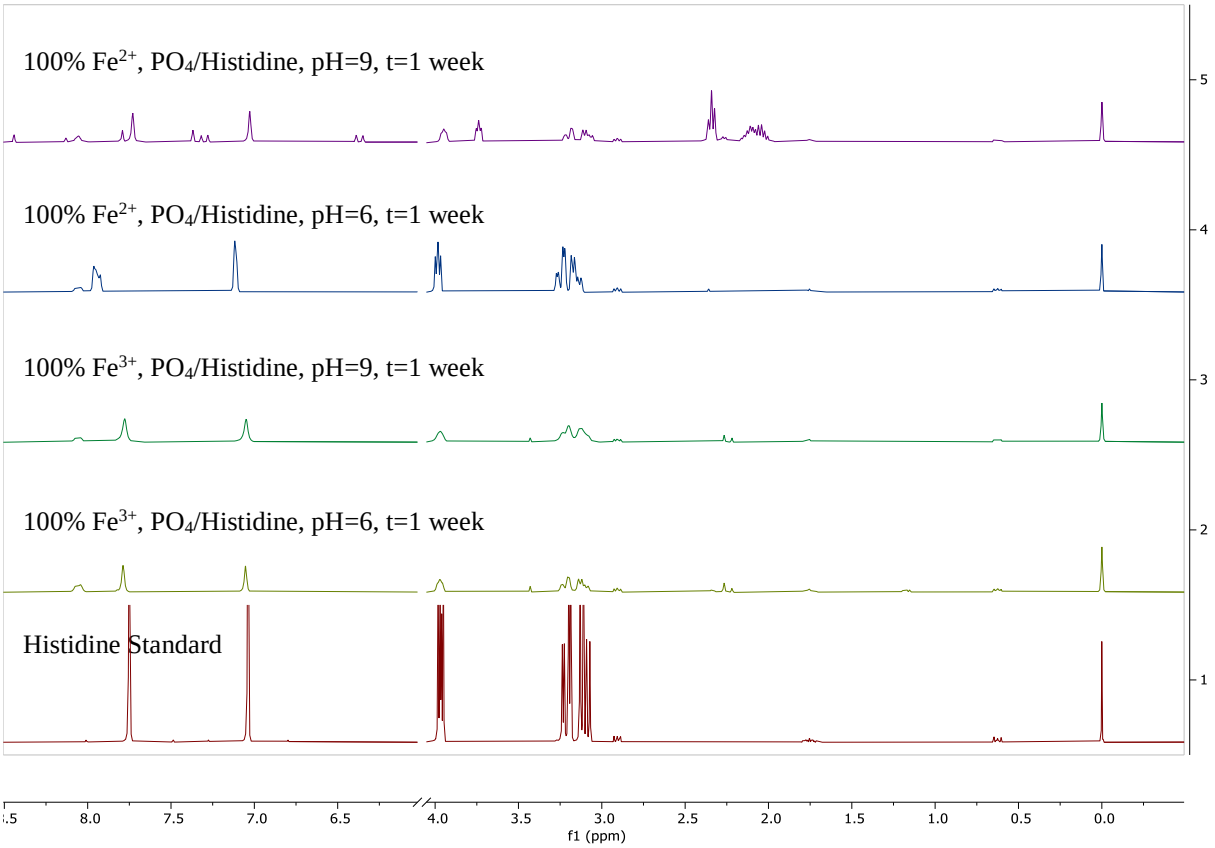

Figure S20. NMR spectra of histidine experiments at every condition tested for t = 1 week and spectrum of histidine standard.

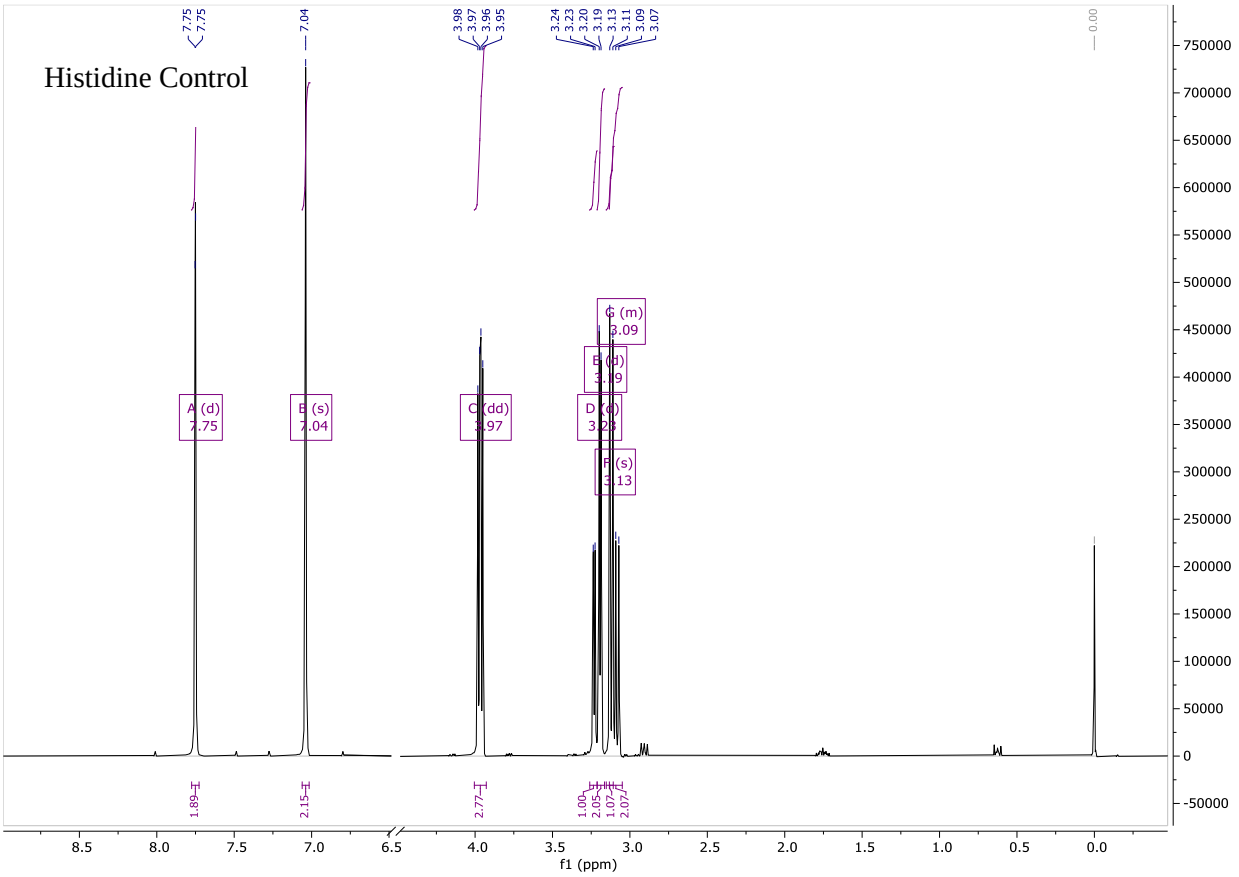

Figure S21. Histidine control in DSS/D_2O.

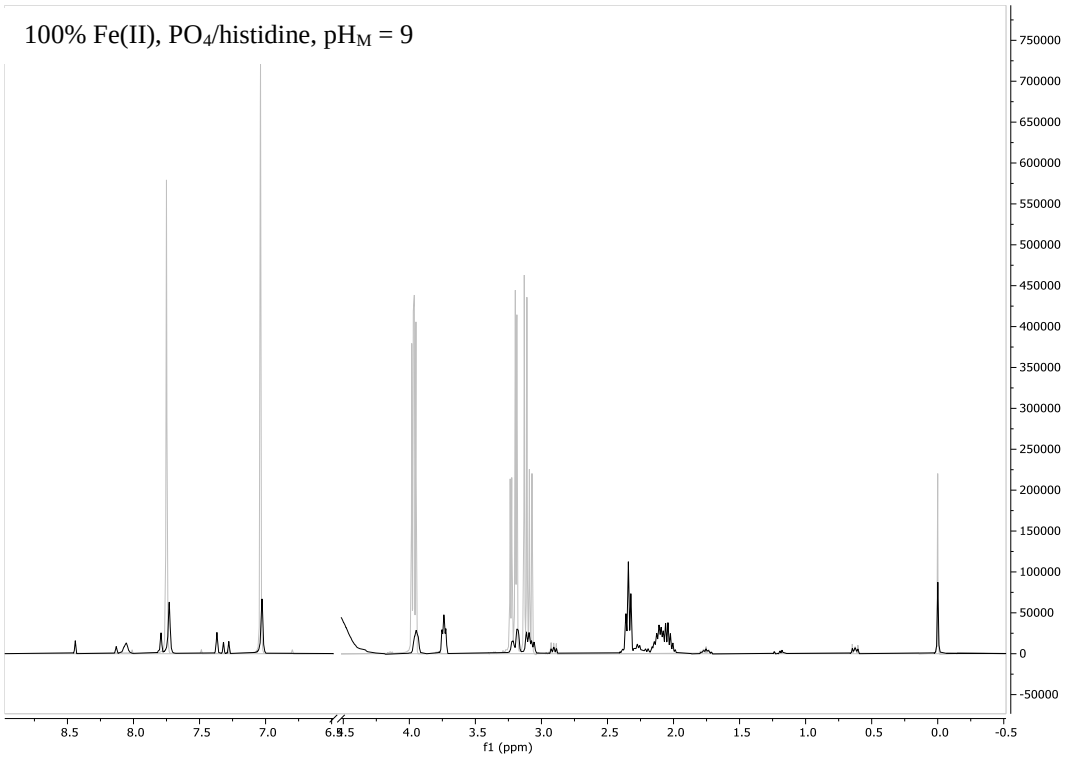

Figure S22. NMR spectra of 100% Fe(II), PO_4/histidine, pH_M = 9 experiment (black) superimposed onto the histidine standard (gray).

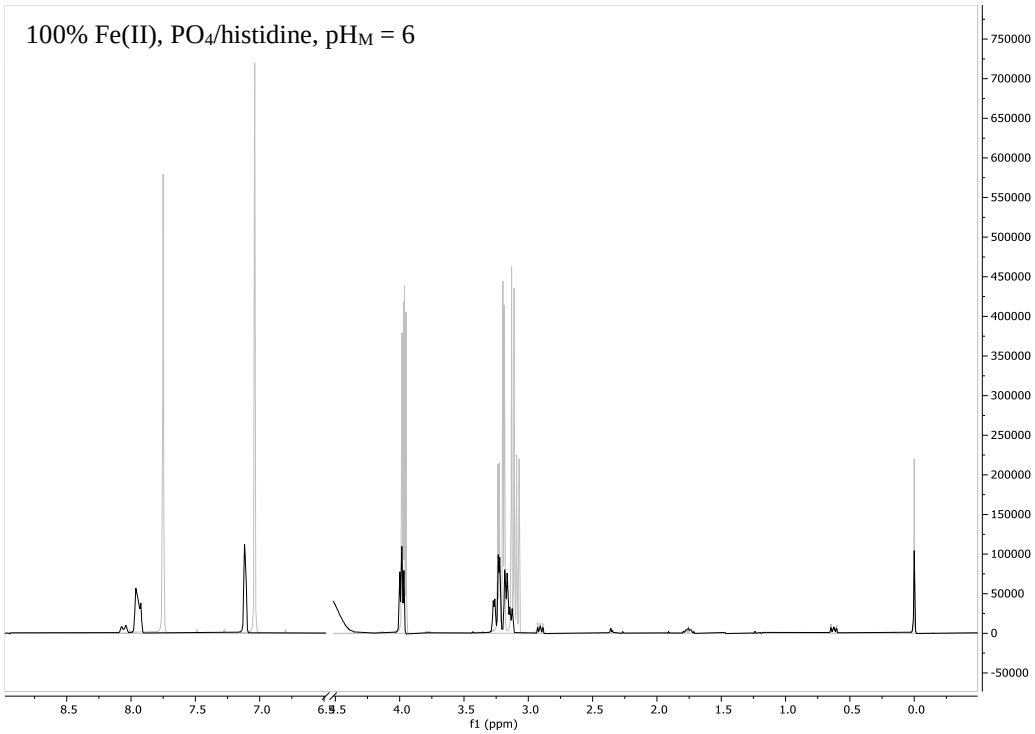

Figure S23. NMR spectra of 100% Fe(II), PO_4/histidine, pH_M = 6 experiment (black) superimposed onto the histidine standard (gray).

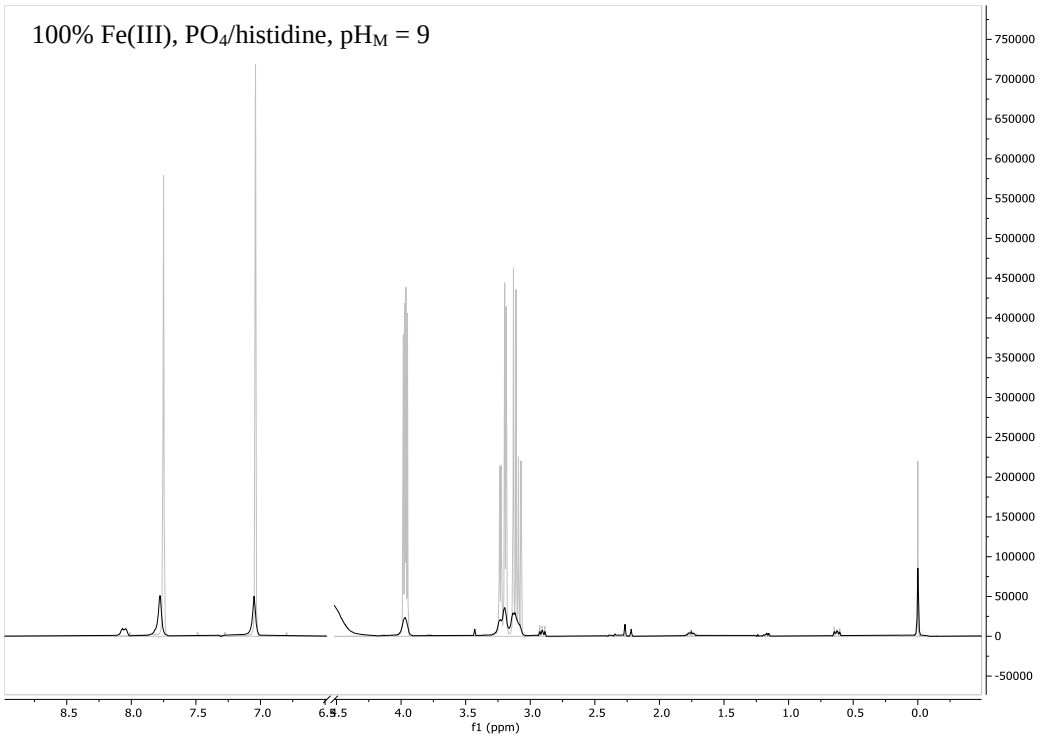

Figure S24. NMR spectra of 100% Fe(III), PO_4/histidine, pH_M = 9 experiment (black) superimposed onto the histidine standard (gray).

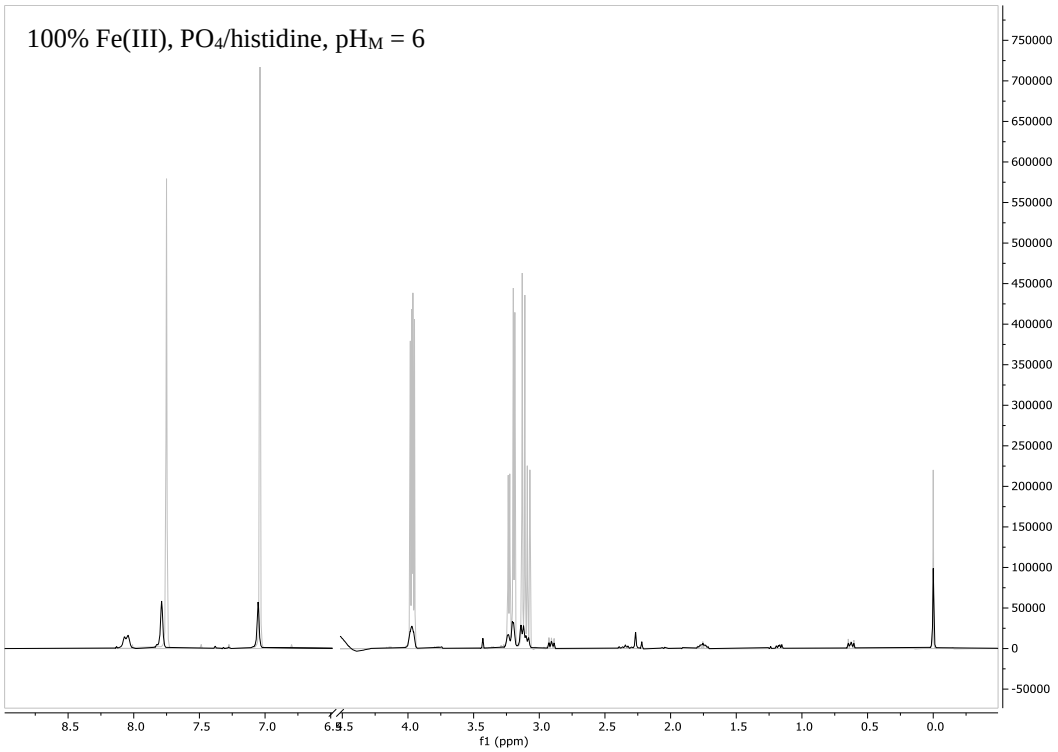

Figure S25. NMR spectra of 100% Fe(III), PO_4/histidine, pH_M = 6 experiment (black) superimposed onto the histidine standard (gray).

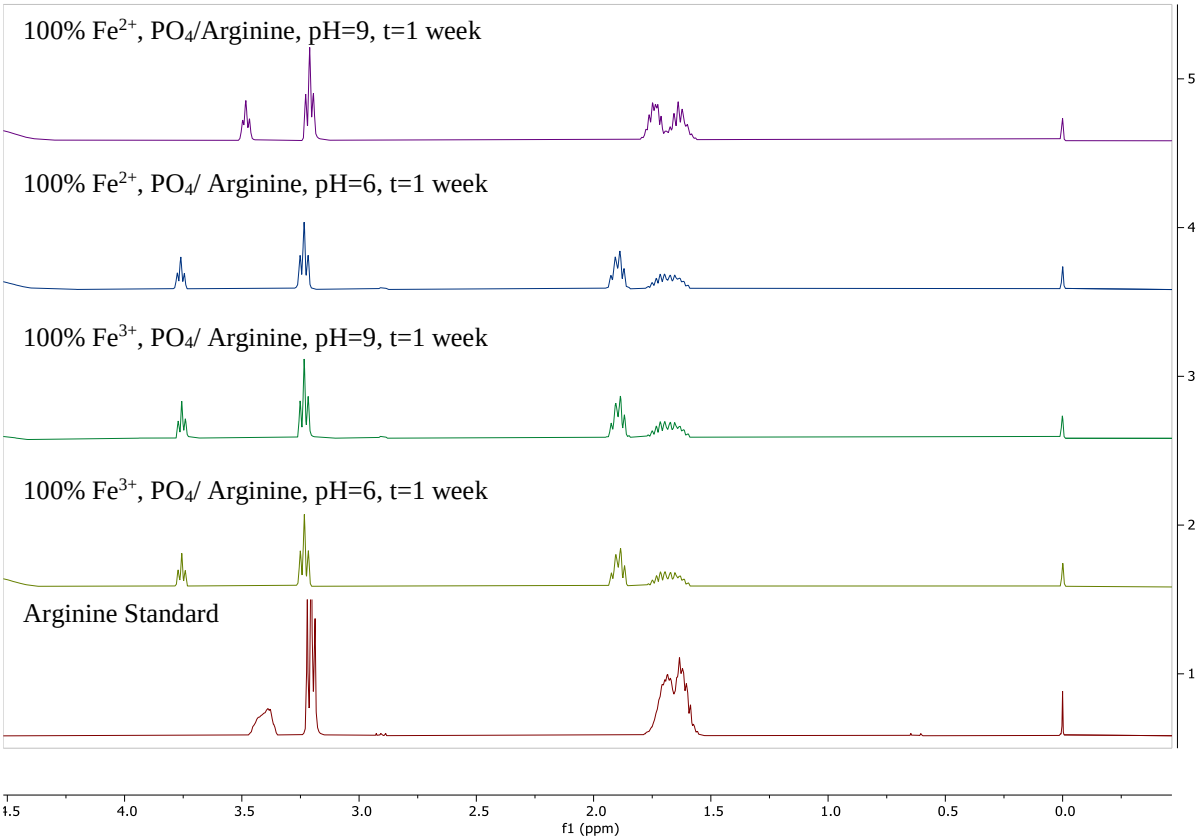

Figure S26. NMR spectra of arginine experiments at every condition tested for t = 1 week and spectrum of arginine standard.

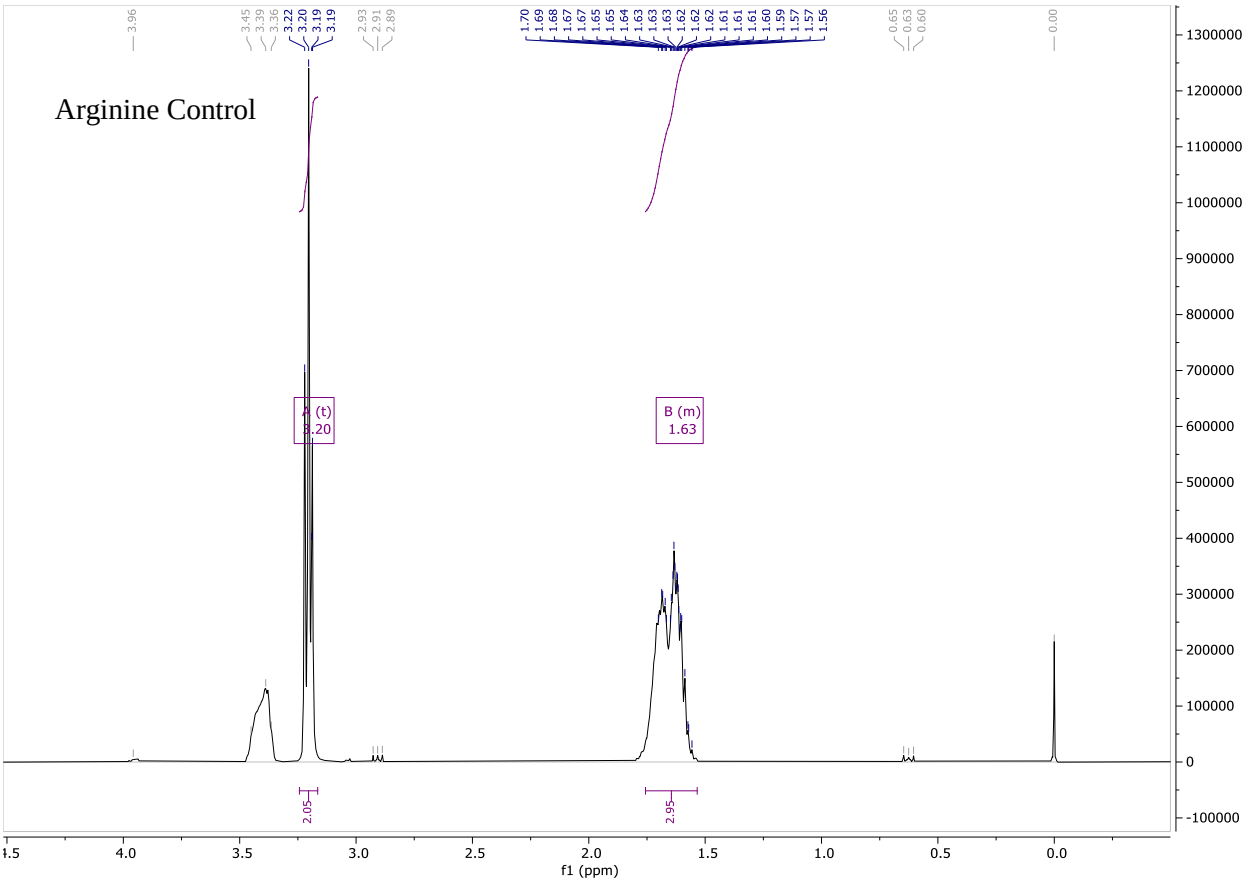

Figure S27. Arginine control in DSS/D_2O.

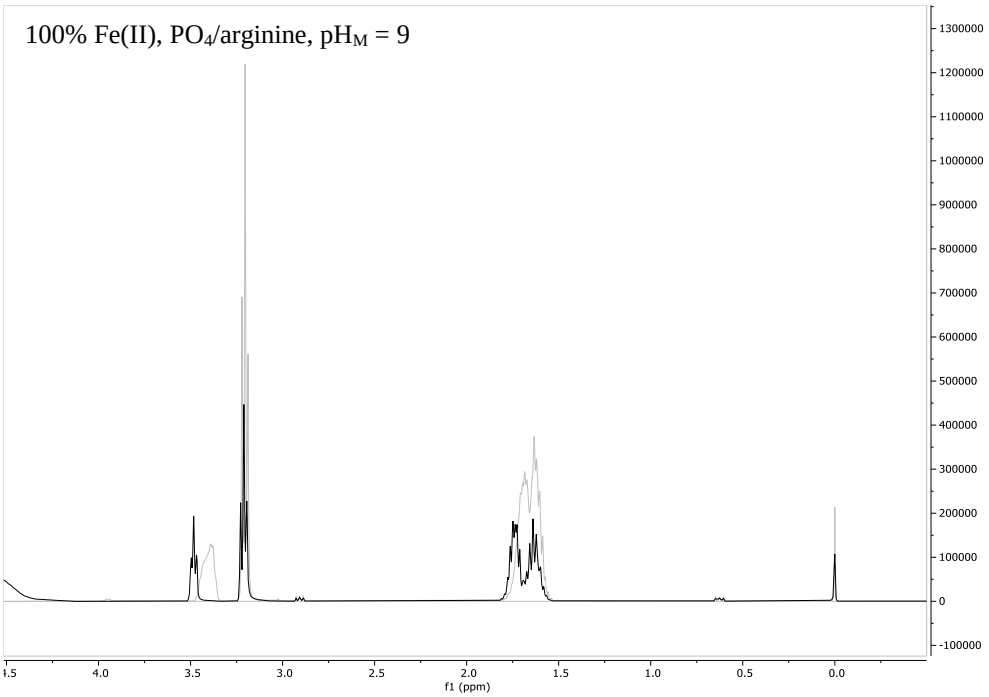

Figure S28. NMR spectra of 100% Fe(II), PO_4/arginine, pH_M = 9 experiment (black) superimposed onto the arginine standard (gray).

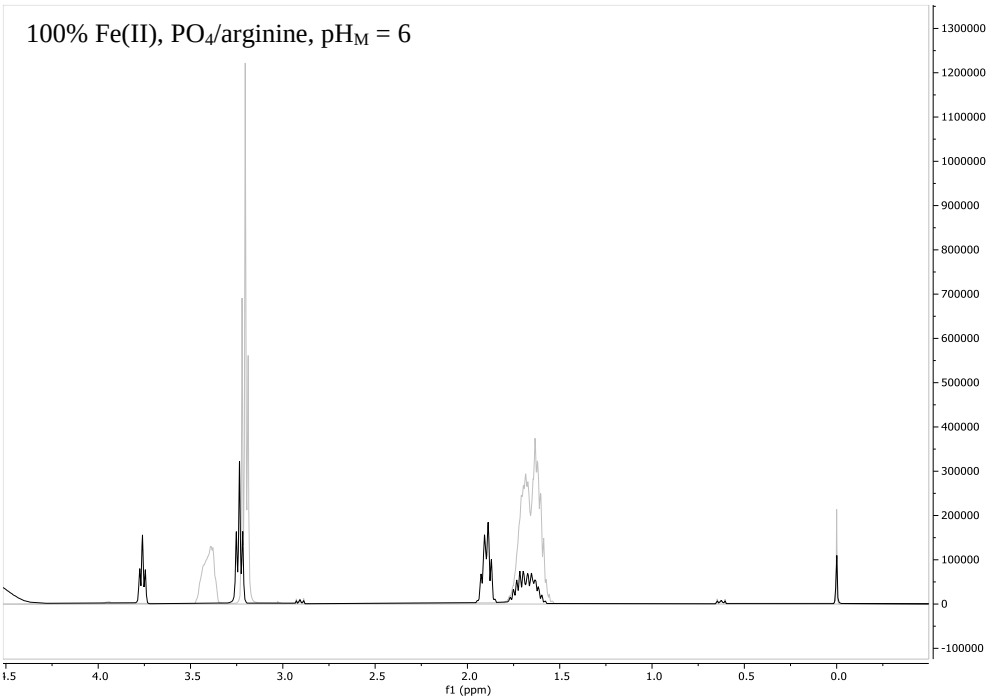

Figure S29. NMR spectra of 100% Fe(II), PO_4/arginine, pH_M = 6 experiment (black) superimposed onto the arginine standard (gray).

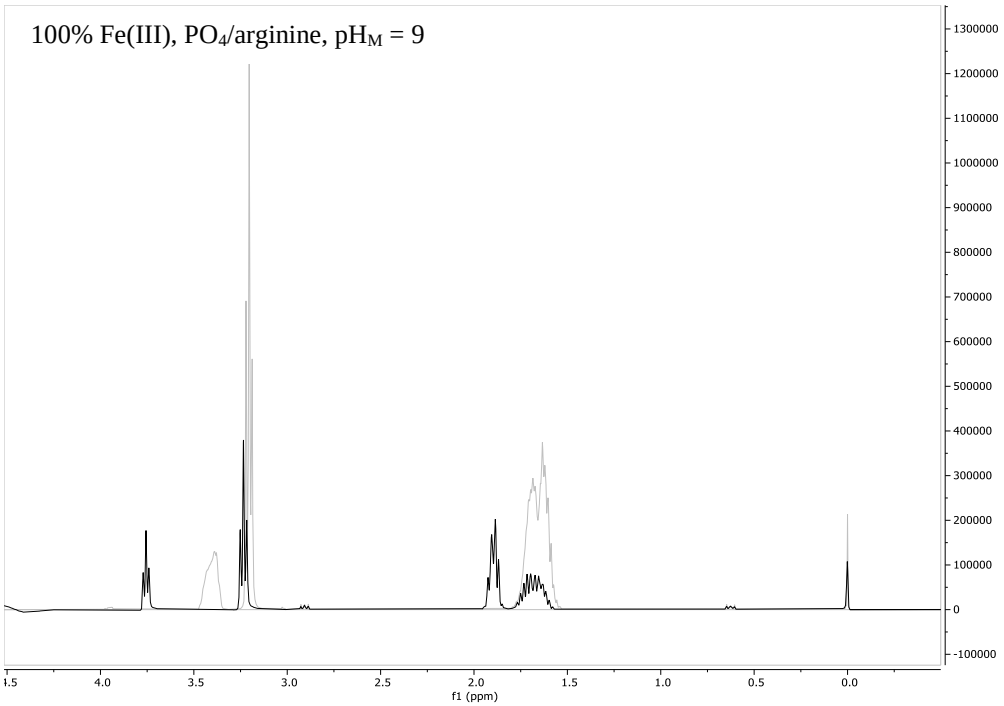

Figure S30. NMR spectra of 100% Fe(III), PO_4/arginine, pH_M = 9 experiment (black) superimposed onto the arginine standard (gray).

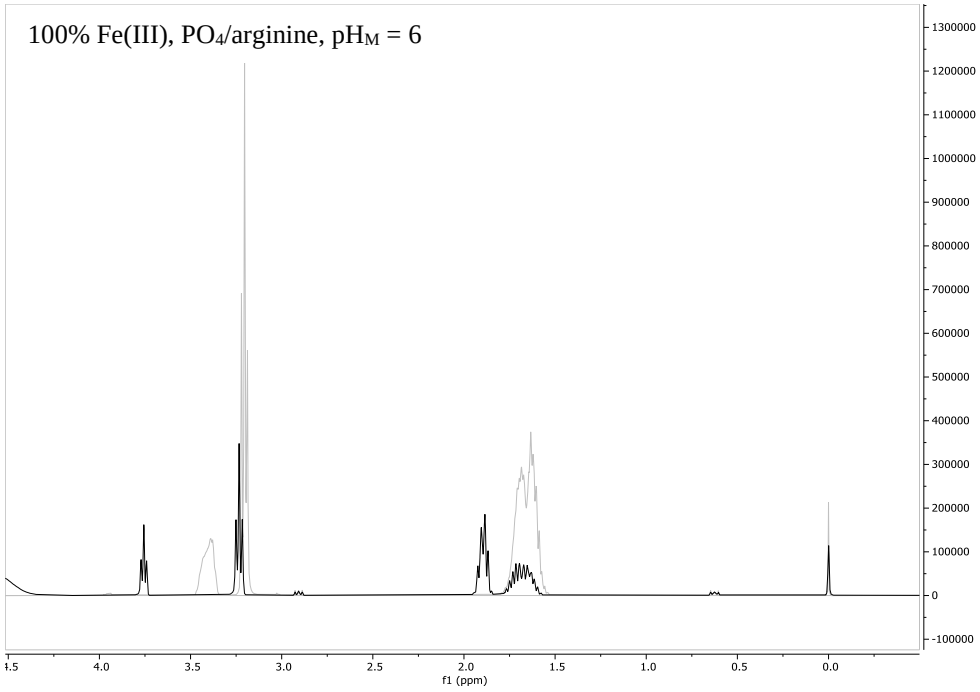

Figure S31. NMR spectra of 100% Fe(III), PO_4/arginine, pH_M = 6 experiment (black) superimposed onto the arginine standard (gray).

STATISTICAL ANALYSIS

Table S3. Results for multiple linear regressions (MLR) of percent phosphate adsorbed by both amino acid and pH.

Experiments	Time[†]	Variable[‡]	Coefficient	*p*- value	Model Evaluation Metrics				
					R^2	adj R^2	F-statistic	df[§]	*p*-value
Ferrous: pH_M 9	All	**Arg vs None**	**31.07**	**<.001***	.70	.70	19.43	4, 34	<.001*
		His vs None	**25.13**	**<.001***					
		Cys vs None	**29.35**	**<.001***					
		pH	**4.88**	**.003***					
Ferrous: pH_M 6	t0	**Arg vs None**	**22.12**	**.04***	.52	.28	2.15	4, 8	.17
		His vs None	9.80	.30					
		Cys vs None	19.26	.08					
		pH	13.61	.32					
	1 d	**Arg vs None**	**22.80**	**.05***	.52	.28	2.14	4, 8	.17
		His vs None	-0.80	.70					
		Cys vs None	3.88	.30					
		pH	10.03	.09					
	7 d	Arg vs None	4.85	.70	.30	-.05	0.87	4, 8	.52
		His vs None	16.67	.19					
		Cys vs None	10.32	.46					
		pH	11.67	.32					
Ferric: pH_M 9 & 6	t0	**Arg vs None**	**40.68**	**<.001***	.46	.36	4.56	4, 21	.008*
		His vs None	17.17	.11					
		Cys vs None	4.42	.67					
		pH	40.99	.24					
	1 d	Arg vs None	9.40	.97	.10	-.07	0.60	4, 21	.67
		His vs None	-0.40	.42					
		Cys vs None	3.02	.90					
		pH	19.57	.67					
	7 d	Arg vs None	1.78	.80	.48	.38	4.91	4, 21	.006*
		His vs None	**23.54**	**.005***					
		Cys vs None	20.30	.23					
		pH	-5.39	.67					

Only organic type and pH were found to be significant variables in initial MLR models; thus, the results reported here are from models only including those variables. [†] When rmANCOVA indicated that there was a significant organic:time interaction (Table 2) MLR was done for each time point. [‡] Amino acids were encoded (value = 1); samples lacking amino acids (i.e. "none") were encoded with value = 0. As such, the effect of adding each of the amino acids is compared to the base level (samples lacking amino acids) and the reported coefficients for each represent the additional percentage of phosphate (on average) adsorbed due to the addition of that amino acid.
[§] Degrees of freedom (df); the first subscript indicates df between groups and the second indicates df within groups.

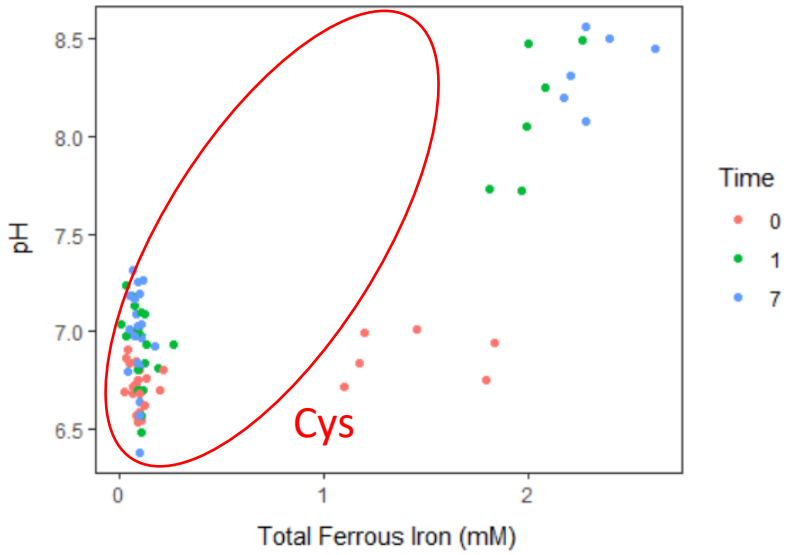

Figure S32. As cysteine reduces ferric to ferrous iron, the pH of the sample increased. Data includes ferric samples with mineral precipitated at both pH_M 6 and 9.

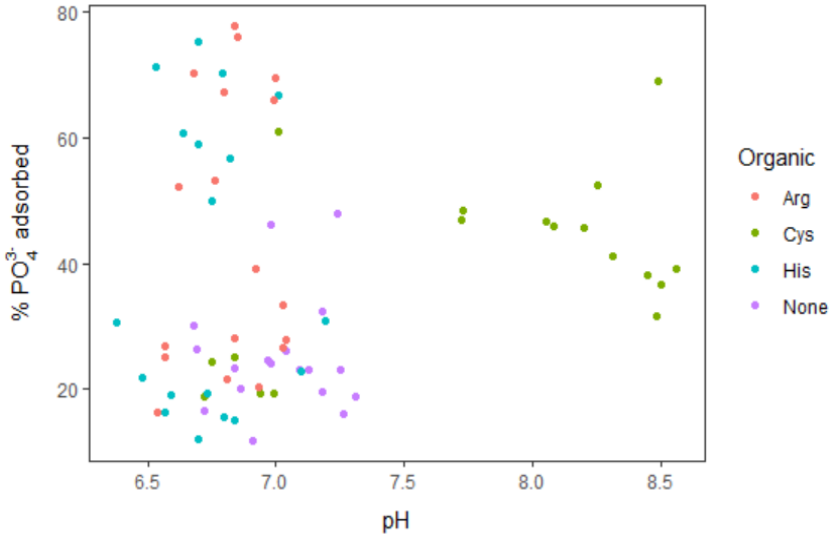

Figure S33. Ferric samples (pH_M 6 and 9) do not show a correlation between pH and percentage of phosphate adsorbed. Note that solutions containing cysteine were at higher pH than those with arginine, histidine, or no amino acid.

REFERENCES

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(3) Sander, J. 2020. Veusz – A Scientific Plotting Package. https://veusz.github.io/ (accessed October 13, 2019).